Synthetic oligonucleotides (oligos) have proven to be effective life science tools for a wide range of applications, from PCR to sequencing. Traditionally, oligo sequences of individual syntheses are performed on a support (typically covalently bound DNA) packed into a synthesis column in varying quantities depending on yield requirements. The wide utility of oligos has driven the development of more scalable technologies to enable the manufacture of oligos en masse in parallel. New applications such as target capture for next-gen sequencing are pushing this need even further, to tens of thousands of sequences. Microarray syntheses have proven a suitable method for parallel oligo synthesis; however, adapting the synthesis chemistry to massive parallel reactions on a solid surface has been challenging. Variations to traditional DPT protection of synthons monomers such as electrophilic photocatalysts have shown success but lack the reaction efficiency and flexibility to incorporate modified monomers. We have developed a microarray synthesis technique to photoreactive acid (PARA) deprotection of standard DPT protected monomers which preserves both the high coupling efficiency and flexibility of traditional solid support oligo synthesis, but enables the massive parallel manufacture of tens of thousands of oligos sequences required for advanced applications. Oligos are synthesized on the microarray chip and then cleaved into solution, ready for use in multiplexing reactions. The cleaved oligos are identical to and can be modified in any way that oligos traditionally synthesized oligos can. Additionally, the ability to incorporate modified terminators, bases or backbone greatly increases the range of the application of these oligos. Microarray-synthesized oligos are now used in various multiplex applications such as target capture for next-gen sequencing, gene synthesis and production of protein coding libraries.

### Targeted Sequencing

Through next-generation DNA sequencing (NGS) provides very high levels of coverage, even on complex genomes, it is still advantageous to reduce the complexity of samples and sequence targets to regions – in particular, when sample numbers are very high and the goal is detection of low prevalent mutations. Several research groups have developed a variety of catch methods for enriching or selectively amplifying subsets of the genome. The key to the micro-mass approach is to achieve both specificity and sensitivity, the ability to multiple many samples and capture large regions of interest, and of course, cost. Recently, several methods have been described. See Table (Appendix C) for a detailed description of each method.

### Targeted Methylation Analysis

Modern biology involves not only the construction of existing genes to edit their functions, but also designing mutation and sequence shifting to create new, functional gene constructs which parsi-moon biotechnologies. One bottleneck for gene synthesis is the cost of making the initial building blocks (oligos) which are assembled together to make genes. Parallel, parallel DNA construction on a large scale requires pools of large numbers of short synthetic oligos.

### Synthetic Biology Applications

Microarray technology provides a fast and economical means for massive parallel synthesis of oligos and the PHASE technology represents a significant advancement in microarray synthesis technology. Large numbers of DNA constructs of designed sequences can be synthesized and then assembled into large molecules. These methods are highly reproducible and eliminate the need to purify individual oligos. The combined cost and speed of library synthesis and subsequent assembly has the potential to greatly increase the rate at which we can design, synthesize, and express new biological function.

### Library Construction Applications

Production of cloned libraries with microarray-based oligo synthesis provides a rapid, high-throughput method for the generation of complex libraries of designed oligo sequences. The flexibility to use complex designed sequences means this approach can address an array of biological questions, such as short barcodes (SB) libraries for high-thoroughput loss-of-function genetics screens and antibodies or other protein coding DNA libraries for diversity studies and directed evolution strategies.

### Genomics Discovery Applications for Microarray Synthesized Oligonucleotide Pools

Technical and economic improvements in microarray technology have enabled these methods to be used in parallel to generate libraries of thousands of oligos. This approach can be used to generate libraries that have been optimized for specific applications, such as microarray or sequencing to detect the increased yield and throughput. In the example here, a mixture of oligo solution, ligase enzyme, and ligation buffer is subjected to multiple rounds of melting and annealing. The ligate product is amplified with a primer set from each region of the target sequence. After amplification, the library is sequenced.

### Library-Free, Parallel, Packaged Oligos (PSPs)

Library construction methods have been demonstrated to be effective in selecting oligos for functional enrichment.

### Proteins Coding / Antibody Library Preparation

Synthetic antibody libraries have proven to be effective tools for drug discovery and development through the use of antibodies to identify antigens against a wide variety of antigens. Large-scale protein synthesis approaches have been developed to generate antibodies quickly and cost-effectively. These approaches rely on the use of immobilized antibody libraries that are typically constructed from large, diverse sets of antibodies.

### References


### OligoMix® Microarray Synthesized Oligos

OligoMix® is a microarray technology that allows for the rapid and cost-effective synthesis of large numbers of defined synthetic oligonucleotides. This technology provides a powerful tool for generating libraries of oligos for use in downstream applications, such as gene synthesis, protein expression, and drug discovery. The key features of OligoMix® include:

- High-throughput, simultaneous synthesis of large numbers of oligos
- Low cost and high yield
- Flexibility in oligo design and sequence
- Compatibility with a wide range of applications

### Conclusion

Microarray-synthesized oligos offer a powerful tool for researchers in the life sciences. The ability to rapidly and cost-effectively generate libraries of large numbers of oligos with defined sequences provides a powerful platform for a wide range of applications, from gene synthesis to drug discovery. The continued development and refinement of microarray technology will likely further expand the range of applications and capabilities of OligoMix® and similar technologies.